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In vitro gas production, rumen fermentation and production performance of steers fed multinutritional prickly pear blocks

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ABSTRACT. The aim of this study was to evaluate the replacement of oat hay exclusively with fermented prickly pear in developed multi-nutritional blocks (MNBs) on steer production efficiency and *in vitro* rumen fermentation parameters. Two experiments were performed: Trial 1 (*in vitro* assay) evaluated *in vitro* fermentation parameters of three MNBs replacing oat hay (MNB0 – 0% prickly pear, MNB1 – 25% prickly pear, MNB2 – 25% fermented prickly pear). Trial 2 (*in vivo* assay) evaluated the effect of MNBs on steer production performance. Both experiments were established as a completely randomized design. The values for proteins, metabolisable energy, gas production, methane and CO_2 were higher in MNB2 ($P < 0.05$) group. Total and individual volatile fatty acids differed between experimental MNBs groups ($P < 0.05$). Mean body weight and mean live-weight gain of steers were increased with MNB supplementation in T2 group by 12 and 37%, respectively. Dry matter digestibility was higher ($P < 0.05$), but methane and CO_2 production in the rumen decreased with MNB supplementation ($P < 0.05$). Replacing 25% of oat hay with fermented prickly pear leaves increases the nutritional quality of MNB2, while improving animal production variables and reducing rumen methane emissions.

Introduction

Over the past forty years, multinutrient blocks (MNBs) have been increasingly used in extensive livestock production systems as a permanent part of animal nutrition. MNB ingredients such as molasses and urea, and forage sources like oat and alfalfa hay provide energy, protein, minerals, agglutinant and fibre, dry distiller grains (DDG) and cottonseed meal in turn supply nutrients that meet the nutritional requirements of cattle, promoting ruminal microbial growth and increasing digestibility and dry matter intake. However, molasses and oat hay are expensive ingredients, thus it is recommended to use non-conventional ingredients that can provide certain nutrients for animal nutrition at low cost (Araiza-Ponce et al., 2020). Some researchers propose the use of prickly pear (*Opuntia ficus-indica*) in arid zones, but although the protein content in prickly pear is low, previous studies reported an increase in prickly pear protein content through the use of solid-state fermentation (SSF) with yeast cultures (Herrera et al., 2017). Prickly pear may be used as an energy ingredient in MNB and as an adhesion promoter like molasses. Nevertheless, prickly pear must be pre-treated with SSF before its incorporation to MNBs. In addition, previous studies have shown that the application of prickly pear in silage development reduced ruminal methane production in *in vitro* assays, thereby contributing to climate change mitigation (González-Arreola et al., 2019). Therefore, the present study aimed to evaluate the substitution of oat hay with prickly pear and fermented prickly pear in MNB production on *in vitro* gas and methane production, ruminal fermentation parameters and production performance in Angus steers.

Material and methods

Study area

Two experimental trials were carried out at the Faculty of Veterinary Medicine and Husbandry of the Durango State Juarez University and the Guadiana Valley Institute of Technology, both institutions located in Durango, Mexico. This study was approved by the Livestock Protection and Promotion of the state of Durango (OF 2019-011-35).

Saccharomyces cerevisiae yeast cultures were donated from the collection of the Durango's Institute of Technology. Prickly pear cladodes (*Opuntia ficus-indica* variety AV6) were randomly harvested from an irrigated field near the university area, while oat hay was acquired from a local store, their chemical composition is presented in Table 1.

Table 1. Chemical composition of oat hay and prickly pear cladodes

Nutrients	Oat hay	PPC
Dry matter, %	90.0	9.3
Crude Protein, %	13.6	5.3
NDF, %	59.8	27.3
ADF, %	32.26	13.5
ME, Mcal/kg	1.8	2.3

PPC – prickly pear cladodes, NDF – neutral detergent fibre, ADF – acid detergent fibre, ME – metabolisable energy

Development of multi-nutritional blocks (MNBs)

Prickly pear was fermented as proposed by Herrera et al. (2017). Three experimental formulations were designed with the inclusion of prickly pear and fermented prickly pear as partial substitution of oat hay in MNB (MNB0 with no prickly pear added, MNB1 with 25% prickly pear and MNB2 with 25% fermented prickly pear, n = 10) (Table 2). To make MNBs, all ingredients were mixed by hand and placed into 20 plastic containers (height 30 cm × diameter 30 cm) and compressed by hand. Subsequently, the freshly pressed MNBs were allowed to dry in the sun for three weeks. The dried MNB samples were then ground to 1-mm particles in a Wiley mill (Arthur H Thomas, Philadelphia, PA, USA) for further laboratory analysis, while complete MNBs were used for animal feeding experiments.

Table 2. Composition of multi-nutritional blocks

Ingredient,% DM	MNB0	MNB1	MNB1
Prickly pear	0	25	0
Fermented prickly pear	0	0	25
Molasses	25	25	25
Oat hay	45	20	20
Minerals	5	5	5
Ground corn	10	10	10
Cement	5	5	5
Limestone	5	5	5
Salt	5	5	5

MNB – multi-nutritional blocks, DM – dry matter, MNB0 – control group without prickly pear addition, MNB1 – group with 25% prickly pear addition, MNB2 – group with 25% fermented prickly pear addition

The content of crude protein (CP), ash and ether extract (EE) of MNBs were determined by standardized procedures (AOAC International, 2019). Neutral detergent fibre (NDF), acid detergent fibre (ADF) and lignin were determined as described by Van Soest et al. (1991). Dry matter digestibility (DDM) was determined using a DaisyII incubator (ANKOM, Macedon, NY, USA) based on dry matter disappearance after 48 h according to the manufacturer's procedures (ANKOM, 2015). Metabolisable energy (ME) of MNB was estimated according to Equation 1 proposed by Menke and Steingass (1988):

$$ME = 2.20 + 0.136\,(GP_{24}) + 0.057\,(CP) + 0.0029\,(EE^2) \qquad (1),$$

where: GP_{24} – gas produced after 24 h of fermentation time (ml/g DM), *CP* – crude protein (% DM), *EE* – ether extract (% DM).

Trial 1 (*in vitro* assay)

Ground samples of each MNB formulation were subjected to *in vitro* analyses to select the best formulation and finally offered to the animals in an *in vivo* experiment.

In vitro gas production

Rumen fluid was collected from two Angus steers fed a 70:30 oat hay-concentrate diet and immediately transported in a thermos to laboratory, it was subsequently mixed, flushed with CO_2 and filtered through four cheesecloth layers (Musco et al., 2016). Approximately 1 g of each experimental MNB was then placed in triplicate in ANKOM glass modules (ANKOM, Macedon, NY, USA) equipped with electronic pressure transducers and incubated with a 2:1 mixture of buffer solutions and ruminal inoculum (González-Arreola et al., 2019). Rumen inoculum incubations were carried out from 0 to 96 h and pressure changes were registered every hour. *In vitro* gas kinetics was fitted into the Gompertz function according to Equation 2:

$$GP = Gmax \times \exp\left[-A \times \exp\left(-k \times t\right)\right] \qquad (2),$$

where: GP – gas production at time t (ml/g DM), $Gmax$ – maximum gas production (ml/g DM), A – lag phase (h), k – constant gas production rate (h^{-1}), t – time (h).

In vitro methane and CO_2 production and fermentation parameters

Methane and CO_2 compositions were measured by incubating approximately 1 g of each treatment in triplicate with a 2:1 mixture of buffer solutions and rumen inoculum in ANKOM glass modules (ANKOM, Macedon, NY, USA) as described by González-Arreola et al. (2019). After 24 h of fermentation, the modules were connected to a portable gas analyser (GEMTM5000, LANDTEC, Dexter, MI, USA) according to the procedures proposed by González-Arreola et al. (2019). To evaluate *in vitro* rumen fermentation parameters, the modules were opened after measuring the gas composition and the pH was immediately measured, the liquid was then filtered and divided into two sub-samples (10 ml each) for treatment with sulphuric acid (300 µl) and metaphosphoric acid (2.5 ml) to evaluate $N\text{-}NH_3$ and volatile fatty acid (VFA) levels, respectively (Galyean, 2010). The samples for ammonia and VFA determination were stored at 4 °C until analyses were completed.

Trial 2 (production performance assay)

This assay was performed using the MNB formulation which performed better in the *in vitro* assays. Twenty-four Angus steers were divided into two groups and randomly placed in individual pens: T1 for animals fed oat hay and ground corn (n = 12), and T2 for animals fed oat hay, ground corn and MNB *ad libitum* (n = 12). Each animal was used as a replicate for each treatment. Dry matter intake (DMI) was restricted to 3% live weight and treatments were offered twice daily (08:00 and 17:00), water was offered *ad libitum*. Dry matter intake was measured on a daily basis by weighing refusals. Average daily gain (ADG) was measured by weighing each animal weekly. MNB intake was estimated by weighing the MNB weekly. The trial lasted 90 days. Prior to the experiment, animals were vaccinated (Bacterina Triple Bovina, Bayer, Berlin, Germany), supplemented with vitamins (Aminoforte L, Agrovet Market, Lima, Peru) and treated for parasites (Ivermectin, Agrovet Market, Lima, Peru). Production variables such as MNB intake, DMI, average daily gain (ADG) and feed conversion (FC) were measured throughout the experiment.

In vivo determination of CH_4 and CO_2 production

The ruminal production of CH_4 and CO_2 was estimated based on DMI of each steer and *in vitro* production for each treatment.

Calculations and statistical analysis

The obtained data on the chemical composition, *in vitro* gas production kinetics, methane and CO_2 production, as well as ruminal fermentation parameters were analysed using a completely randomised design. Three multinutritional blocks for each treatment were randomly selected and subjected to each analysis as a replicate. The obtained data from animal performance were analysed using a completely randomised design with a generalised linear model. Each animal was treated as an experimental unit in the *in vivo* experiment, considering the treatments as fixed effects and random errors associated with each observation. Initial weight was introduced as a covariate using the procedures of SAS (SAS Software ver. 9.4, SAS Institute; Cary, NC, USA). Means of treatments were compared using the Tukey test for both trials ($P < 0.05$).

Results and discussion

Trial 1 (*in vitro* assay)

The inclusion of prickly pear reduced dry matter by 13% in MNB1 and by 33% in MNB2. In addition, the incorporation of fermented prickly pear increased the protein content by 69% in case of MNB2, while it reduced the protein content by 27% in MNB1. Otherwise, prickly pear supplementation reduced NDF by more than 50% in both nutritional blocks (MNB1 and MNB2). ADF and lignin fractions also decreased with prickly pear inclusion in both blocks (by 50 and 30%, respectively). In addition, ME was similar between MNB0 and MNB1, but different from MNB2, in which the addition of fermented prickly pear increased ME by 37% (Table 3).

The inclusion of prickly pear reduced dry matter (DM) in MNBs due to differences in DM in oat hay, as the DM content in oat hay is higher.

Table 3. Effect of prickly pear inclusion into multinutritional blocks on chemical composition

Nutrients, % DM	MNB0	MNB1	MNB2	SEM	*P*-value
Dry matter	92.5 ± 0.02^a	80.4 ± 1.18^b	73.0 ± 3.0^c	0.55	0.0325
Ash	26.4 ± 0.57^a	24.0 ± 0.06^b	19.2 ± 0.12^c	0.13	0.0001
Crude protein	12.6 ± 0.37^b	9.1 ± 0.26^c	21.3 ± 0.55^a	0.42	0.0001
Ether extract	0.82 ± 0.05^b	1.6 ± 0.08^a	2.0 ± 0.28^a	0.15	0.0010
NDF	35.2 ± 2.32^a	17.5 ± 0.57^b	14.5 ± 0.57^c	0.42	0.0001
ADF	20.4 ± 0.82^a	9.6 ± 0.14^b	9.1 ± 0.08^b	0.82	0.0002
Lignin	2.6 ± 0.60^a	1.8 ± 0.05^b	1.8 ± 0.08^b	0.20	0.0001
DMD	64.6 ± 1.34^c	73.28 ± 1.07^b	81.7 ± 0.97^a	0.93	0.0001
ME, Mcal/kg DM	2.9 ± 0.60^b	3.2 ± 0.04^b	4.0 ± 0.06^a	0.04	0.0002

MNB – multi-nutrient blocks, NDF – neutral detergent fibre, ADF acid detergent fibre, ME – metabolisable energy, MNB0 – control group without prickly pear addition, MNB1 – group with 25% prickly pear addition, MNB2 – group with 25% fermented prickly pear addition, SEM – standard error of the mean; ^{abc} – means within a row with different superscripts are significantly different at $P < 0.05$

Fibre content in prickly pear was as high as in oat hay, while the proportion of non-structural carbohydrates contained in prickly pear was lower than the proportion in oat hay. Del Razo et al. (2015) reported similar results comparing the chemical composition of oat hay and prickly pear. The current study reported a protein content of 5.3% in contrast to the previously reported 4.5% in the same prickly pear variety (var AV6) (Herrera et al., 2017). In addition, the protein concentration in oat hay was 11%, which explained the lower protein content in MNB1 compared to MNB0. The cladodes used in MNB2 were subjected to the SSF process using yeast cultures, which increased the protein content due to the proliferation of yeast cells increasing overall protein levels (Herrera et al., 2017). In addition, ME was estimated by equation that uses variables such as PC, EE and gas production parameters. For this reason, the estimated crude fat content in prickly pear was higher than in oat hay. Therefore, an increase in ME was expected with increasing ether extract and gas production for MNB1 and MNB2 (Table 3).

In vitro gas production and rumen fermentation

Maximum gas production (Gmax) (Table 4) increased by 13% when prickly pear was added to MNB1, while it increased by 37% when fermented prickly pear was included in MNB2. The same trend was observed in gas production after 24 h of fermentation (GP_{24}). Hence, the inclusion of fermented prickly pear increased GP_{24} by 52% in MNB2 compared to MNB0, while in MNB1, it resulted in similar values to those obtained for MNB0. Similarly, the constant gas production rate (k) reached higher values for MNB1 and MNB2 compared to MNB0, as this variable increased by more than 35% for both units when prickly pear was included in the formulation. Lag phase (A) increased by 11% with the addition of fermented prickly pear in MNB2 compared to MNB0 (Table 4). Methane and CO_2 levels were higher in MNB2 – the inclusion of fermented prickly pear increased these variables by more than 50% compared to MNB0. However, no differences in the CH_4:CO_2 ratio were observed between the blocks. Gas production was also higher in MNBs, as a result of prickly pear addition. The fibre content contained in prickly pear, especially ADF, was lower than in oat hay. In addition, non-structural carbohydrate levels were shown to be higher in oat than in oat hay, which promoted gas formation and improved organic matter fermentation by non-fibrous microorganisms. Murillo-Ortiz et al. (2019) recorded an increase in gas production by about 30% when

Table 4. Effect of fermented prickly inclusion into multinutritional blocks on *in vitro* gas production

	MNB0	MNB1	MNB2	SEM	*P*-value
Gmax, ml	77.3 ± 0.88^c	87.7 ± 0.14^b	106.1 ± 0.95^a	0.61	0.0001
A, h	2.34 ± 0.006^b	2.4 ± 0.05^b	2.6 ± 0.05^a	0.04	0.0234
k, h^{-1}	0.11 ± 0.01^b	0.16 ± 0.005^a	0.15 ± 0.003^a	0.006	0.0064
GP_{24}, ml	66.8 ± 0.65^b	70.5 ± 0.50^b	101.8 ± 0.67^a	1.32	0.0023
Methane, ml/g DM	8.3 ± 0.55^b	8.8 ± 0.52^b	13.1 ± 0.28^a	0.65	0.0024
CO_2, ml/g DM	54.4 ± 2.2^b	57.7 ± 1.8^b	83.5 ± 1.2^a	1.62	0.0015
CH_4:CO_2 ratio	0.14 ± 0.001^a	0.15 ± 0.016^a	0.15 ± 0.012^a	0.008	0.123

Gmax – maximum gas production, A – lag phase, k – constant rate of gas production, GP_{24} – gas production after 24 h of fermentation, MNB0 – control group without prickly pear addition, MNB1 – group with 25% prickly pear addition, MNB2 – group with 25% fermented prickly pear addition, SEM – standard error of the mean; ^{abc} – means within a row with different superscripts are significantly different at $P < 0.05$

MNBs were supplemented with fermented prickly pear and fed to steers. Gas production after 24 h of fermentation (GP_{24}) represented more than 80% of the total amount of gas produced after 96 h of fermentation (Gmax), which was consistent with the results published by Vázquez-Mendoz et al. (2017). Similarly, Zhang et al. (2015) observed higher GP_{24} values when they increased the starch fraction by reducing the fibre fraction in *in vitro* tests. However, the same authors did not observe any changes in the delayed phase and gas production rate and determined similar protein content (approximately 13%) between experimental treatments. In the present study, the protein content was increased above 20% in MNB2. These changes could promote the activity of proteolytic bacteria, which in turn could increase the delay phase (parameter A). The addition of fermented prickly pear to MNBs did not show any effect on pH (Table 5), while its inclusion increased N-NH_3 by 26% in MNB2 and reduced this variable by more than 16% in MNB1 compared to MNB0. On the other hand, similar results were observed for MNB1 and MNB2 with respect to acetic acid levels as its concentrations decreased when prickly pear was added to the preparation. In contrast, propionic and butyric acid contents increased along with the addition of prickly pear to MNB1 and MNB2 compared to MNB0, but similarly no differences were observed between both experimental blocks. Propionic acid levels increased by more than 130% in MNB1 and MNB2, butyric acid levels were also increased by over 55% after prickly pear addition to MNB1 and MNB2. The Ac:P ratio decreased, while total VFA (TVFA) increased with the inclusion of prickly pear in both MNB1 and MNB2 (Table 5).

Table 5. Effect of fermented prickly inclusion into multinutritional blocks on *in vitro* ruminal fermentation parameters

Parameter	MNB0	MNB1	MNB2	SEM	*P*-value
pH	6.9 ± 0.010[a]	6.9 ± 0.015[a]	6.9 ± 0.03[a]	0.025	0.254
N-NH_3, mg/dl	6.1 ± 0.16[b]	5.1 ± 0.08[c]	7.7 ± 0.22[a]	0.094	0.0005
Acetic acid, %	72.8 ± 0.38[a]	53.7 ± 1.07[b]	52.0 ± 0.12[b]	0.382	0.0010
Propionic acid, %	12.0 ± 0.12[b]	28.7 ± 0.59[a]	30.8 ± 0.68[a]	0.204	0.005
Butyric acid, %	7.9 ± 0.14[b]	12.7 ± 0.11[a]	12.4 ± 0.29[a]	0.116	0.05
Ac:P ratio	6.0 ± 0.09[a]	2.2 ± 0.07[b]	1.6 ± 0.00[c]	0.040	0.0025
TVFA, Mm	6.7 ± 0.03[c]	9.4 ± 0.10[b]	11.1 ± 0.47[a]	0.161	0.0001

Ac:P – acetate:propionate, TVFA – total volatile fatty acids, MNB0 – control group without prickly pear addition, MNB1 – group with 25% prickly pear addition, MNB2 – group with 25% fermented prickly pear addition, SEM – standard error of the mean; [abc] – means within a row with different superscripts are significantly different at $P < 0.05$

Thus, the gas production values after 24 h of fermentation were consistent with previous reports by Vazquez-Mendoza et al. (2017), who evaluated prickly pear as animal feed. As already mentioned, more than 50% of the total amount of gas was produced during the first 24 h, thus the lowest fibre values (i.e., NDF and ADF) in MNB1 led to higher gas production. These results were similar to those reported by Khan et al. (2021). On the contrary, higher NDF values and lower protein values in MNB0 resulted in reduced gas production. Moreover, higher methane and CO_2 values were determined in the current study when fermented prickly pear (MNB2) was included in the blocks. However, these increases were more likely associated with higher gas production (Gmax and GP24) than with increased methane synthesis. No changes were observed in the CH_4:CO_2 ratio, which directly correlates with changes in rumen methane synthesis, higher values of this ratio suggest increased methane synthesis by CO_2 reduction (Murillo-Ortiz et al., 2018). Thus, changes in methane synthesis are more likely related to alterations in the propionate fraction (Ferraro et al., 2009).

Previous studies have reported an increase in the content of TVFA and individual fatty acids, mainly propionate, with incremental doses of non-structural carbohydrates in the substrate (Zhang et al., 2015). Murillo-Ortiz et al. (2019) observed similar results in individual VFAs and TVFAs as in the present work. Changes in N-NH_3 concentrations were consistent with alterations in the protein fraction. Higher protein content indicate higher enzymatic activity of proteases, thus, a higher protein content would lead to a higher ammonia concentration. As shown in this study, higher ammonia concentrations were observed in MNB2. Similar results were reported by Zhang et al. (2015) who improved proteolytic activity by reducing the fibre fraction. In addition, Murillo-Ortiz et al. (2019) observed an increase in total bacterial count, which ultimately elevated rumen ammonia levels.

Trial 2 (*in vivo* assay)

No differences in initial animal body weight were observed between individuals supplemented with MNB2 and those that did not receive it.

Table 6. Effect of supplementation of multinutritional blocks with fermented prickly pear on performance parameters of Angus steers

Variables	T1	T2	SEM	*P*-value
Initial weight, kg	100.3 ± 2.12[a]	101.1 ± 3.25[a]	0.55	0.234
Final weight, kg	173.4 ± 3.78[b]	195.8 ± 4.21[a]	0.73	0.005
ADG, kg/d	0.8 ± 0.12[b]	1.1 ± 0.28[a]	0.01	0.0008
DMI, kg DM/d	4.1 ± 0.14[a]	4.3 ± 0.12[a]	0.03	0.0009
FC, kg DM/kg live weight	5.1 ± 0.57[a]	4.3 ± 0.52[b]	0.39	0.0006
DM digestibility, %	63.9 ± 3.88[a]	65.4 ± 2.96[a]	2.81	0.650
Methane, g/d	35.3 ± 0.38[a]	24.6 ± 2.52[b]	0.65	0.001
CO_2, g/d	515.3 ± 0.52[a]	376.2 ± 5.74[b]	6.27	0.0001

ADG – average daily gain weigh, DMI – dry matter intake, FC – feed conversion, CO_2 – carbon dioxide, T1 – treatment 1 for Angus steers fed oat hay and ground corn without multinutritional block supplementation, T2 – treatment 2 for Angus steers fed oat hay and ground corn supplemented *ad libitum* with MNB2, SEM – standard error of the mean, [ab] – means within a row with different superscripts are significantly different at $P < 0.05$

MNB2 uptake was recorded weekly and averaged 634 g/day (included in DMI results in Table 6). The final mean body weight of MNB2-fed animals increased by about 12%. In addition, average daily live weight gain (ADG) increased with supplementation, as animals supplemented with MNB2 had higher ADG by 37%. As a result, MNB2 supplementation reduced feed conversion (FC) by 15%. However, no changes in DMI were observed between treatments. Moreover, methane and CO_2 production in the rumen differed between treatments (Table 6). As observed in Trial 1, better chemical composition results were achieved with MNB2 supplementation. Therefore, MNB2 was selected for *in vivo* feeding tests using Angus bulls (Trial 2). As mentioned above, the higher performance of bulls fed MNB2, compared to the control group, could be due to this supplementation, as it provided more nutrients in relation to grass-based feed. The increase in average body weight and feed efficiency could also be related to improved nutrient and mineral availability. Easily fermentable energy sources in the form of molasses and starch could increase the utilisation of urea from MNB2 by microbes in the rumen (Khalil et al., 2015). A study on rumen characteristics (Zarah et al., 2014) showed that the inclusion of multi-nutritional blocks in the diet of crossbred steers led to a significant improvement in DM degradation in the rumen, and thus improved animal performance. On the other hand, ADG in the present study was higher in animals supplemented with MNB2 (Table 5). However, the reported results for DMI suggested that no changes occurred in this variable. Mendoza et al. (2017) found that block composition could modify intake, indicating the presence of interactions between nutrients in the block and the basal diet. However, intake was not improved in the current study. Nevertheless, mean body weight and mean live weight gain increased, which was associated with higher digestibility (Sanz-Sáez et al., 2012). Murillo-Ortiz et al. (2019) found that supplementation with MNB containing fermented prickly pear improved rumen digestion and apparent digestibility of dry matter and organic matter, while significantly reducing rumen retention time. This was unexpected as there were no changes in DMI between individual diets, suggesting that cattle may have opted for a lower protein diet. However, due to the high CP and non-fibrous carbohydrate (NFC) values in the concentrate, it would not have been possible to select a low protein and high NFC diet at the same time. In addition, ADG in the current study was improved by 37%. This was consistent with the results of Graillet-Juarez et al. (2017), who reported ADG of 494 g in steers supplemented with a multi-nutritional block compared to 398 g in control. In turn, methane and CO_2 production in the rumen in animals supplemented with MNB2 decreased by 30 and 27%, respectively. The *in vivo* CH_4 and CO_2 values (per kg of dry matter consumed) indicated that the consumption of blocks and their additives was sufficient to modify rumen fermentation. If DMI was the same for both treatments, but ADG was higher in animals receiving MNB2, it could be concluded that MNB2 supplementation could shorten the time required to reach the expected average body weight, reduce daily emissions and be an alternative feed limiting global warming. Most studies focus on daily data, but it is important to consider the effects and their impact on global warming over time.

Conclusions

Substituting 25% oat hay with fermented prickly pear leaves in MNB processing improved *in vitro* protein content and fermentation properties by increasing total volatile fatty acid levels and gas production without affecting rumen methane and carbon dioxide synthesis. Furthermore, the addition of the proposed MNB2 improved ADG and final weight of steers by 15 and 12%, respectively, and reduced daily methane and CO_2 emissions. MNB supplementation is therefore an alternative to animal feed that has the potential to reduce methane and carbon dioxide emissions.

Conflicts of Interest

The Authors declare that there is no conflict of interest.

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